Estimation and Stability of Added DL-Methionine in Mixed Feeds

EDWARD L. ROHDENBURG and HANS R. ROSENBERG

Stine Laboratory, E. I. du Pont de Nemours & Co., Inc., Newark, Del.

To test for added DL-methionine in mixed feeds containing ingredients used in commercial animal feeds, the DL-methionine is extracted from the feed with water and the amount present in the extract is determined microbiologically. Using *Streptococcus faecalis* (ATCC 9790), ingredients normally found in commercial poultry diets—e.g., antibiotics, arsanilic acid, diphenyl-*p*-phenylenediamine, fats, and a coccidiostat—do not interfere with the assay. With this procedure DL-methionine in mixed feeds, stored under warehouse conditions, has been found stable for more than a year.

DL-METHIONINE WAS THE FIRST ESSENTIAL AMINO ACID PRODUCED on a large scale and made available at low cost. The nutritional benefits derived from its proper use (*1*) have brought about wide-spread supplementation of many animal feeds, particularly broiler and turkey starter rations.

The commercial use of DL-methionine has focused attention on the problem of the assay of this amino acid when added to feed. For the determination of the total or bound amino acid of the diet, microbiological assay procedures, after hydrolysis of the sample, have been found satisfactory, and are in wide use. Methods to determine free amino acids in mixed feeds have not been published as far as is known. Such a procedure for the determination of DL-methionine would be of benefit to feed manufacturers and feed control officials, as well as the ultimate user. Of equal importance is the question of the stability of the added amino acid after storage. Therefore, the method used in this laboratory during the past 3 years for the estimation of added DL-methionine in animal rations is presented, as well as some data which show that DL-methionine in mixed feeds may be considered stable for all practical purposes.

Estimation of DL-Methionine in Feeds

The procedure for the determination of DL-methionine in feeds is to extract the amino acid with water, and to determine the DL-methionine in the water extract by a microbiological assay. Other procedures may be satisfactory but have not been investigated.

Preparation of Sample. To obtain a uniform sample for assay purposes, it is generally advantageous to regrind a representative amount of the feed mixture. Two grams of the ground feed are weighed into a stoppered 200-ml. flask. One hundred milliliters of distilled water at room temperature (20° to 25° C.) are added. The mixture is shaken in a shaking machine at moderate speed (about 70 strokes per minute) for 5 minutes, and is then filtered through a fine paper such as Whatman's No. 42. If necessary, the pH of the filtrate is adjusted to that of the medium, pH 6.8.

Methionine Assay. The assay for DL-methionine in the filtrate may be carried out by any of the established

Table I. DL-Methionine Content of Various Poultry Feeds

	Other Additives per Ton	L-Methionine Activity, %			DL-Methionire, %	
		In methionine supplemented diet	In unsupplemented diet	Found due to added DL-methionine	Found[a]	Added
Diet A	Tallow (50 lb.)	0.032	0.005	0.027	0.049	0.05
Diet B	Grease (50 lb.)	0.032	0.005	0.027	0.049	0.05
Diet B	Penicillin (4 g.)	0.039	0.008	0.031	0.056	0.05
Diet B	Aureomycin (10 g.)	0.033	0.006	0.027	0.049	0.05
Diet B	Terramycin (10 g.)	0.039	0.006	0.033	0.060	0.05
Diet B	Nitrophenide (92 g.)	0.038	0.006	0.032	0.058	0.05
Diet B	Arsanilic acid (92 g.)	0.036	0.004	0.032	0.058	0.05
Diet C	DPPD (92 g.)	0.114	0.005	0.109	0.198	0.20

[a] Corrected for response of D-methionine (see text).

Table II. Stability of DL-Methionine in Poultry Rations

	Conditions of Storage	Time of Storage, Months	L-Methionine Activity, %			DL-Methionine, %	
			In supplemented diet	In unsupplemented diet	Found due to added DL-methionine	DL-methionine found[a]	DL-methionine added
Diet D	Room temperature	1	0.037	0.010	0.027	0.049	0.050
	Room temperature	3	0.025	0.002	0.023	0.042	0.050
	Room temperature	10	0.028	0.003	0.025	0.045	0.050
Diet E	Room temperature	3	0.041	0.008	0.033	0.060	0.0625
	Room temperature	6	0.034	0.002	0.032	0.058	0.0625
	Room temperature	13	0.046	0.006	0.040	0.072	0.0625
Commercial	Warehouse	0	0.040	0.010	0.030	0.054	0.050
	Fibrepak carton	6	0.031	0.002	0.029	0.053	0.050
	Cotton bag	6	0.029	0.002	0.027	0.049	0.050
	Burlap bag	6	0.031	0.002	0.029	0.053	0.050
	Fibrepak carton	14	0.031	0.002	0.029	0.053	0.050
	Cotton bag	14	0.031	0.002	0.029	0.053	0.050
	Burlap bag	14	0.031	0.002	0.029	0.053	0.050
Commercial	Room temperature	0	0.040	0.010	0.030	0.054	0.050
	37° C. oven	3 days	0.040	0.010	0.030	0.054	0.050
	50° C. oven	5 days	0.035	0.005	0.030	0.054	0.050
	100° C. oven	3 days	0.006	0	0.006	0.011	0.050

[a] Corrected for response of D-methionine (see text).

procedures. In this laboratory the microbiological procedure described by Stokes and coworkers (*3*) using *Streptococcus faecalis* (ATCC 9790) has proved to be satisfactory. For this purpose, the filtrate of the water extract is measured into culture tubes in increasing amounts between 0.5 and 5.0 ml., and then made up to a volume of 5 ml. with water before adding 5 ml. of Stokes' methionine-free medium. L-Methionine is used as the standard and tubes containing it are similarly prepared in duplicate in a range between zero and 60 γ per tube. The tubes are then sterilized and the absorbances determined spectrophotometrically—e.g., with a Coleman Junior spectrophotometer. A slight precipitate or cloudiness may occasionally occur in some samples but this usually disappears on shaking, and does not interfere with the assay. Following inoculation, the tubes are incubated overnight (16 to 18 hours) and the absorbances measured. The difference between the absorbances found before and after incubation is used for interpretation. With this procedure, correction is made for possible cloudiness of the samples or variation in thickness of the glass of the assay tubes.

Methionine Response of Broiler Diets Not Supplemented with DL-Methionine. When mixed feeds without any added DL-methionine are extracted and assayed according to this procedure, a small response is invariably obtained, suggesting the presence of traces of free methionine or of an extractive causing a methionine response in the microbiological test. The data presented in Table I and II show that this response may correspond to amounts varying from 0.002 to 0.010% L-methionine. This slight response does not interfere with the determination of the DL-methionine added to animal feeds. However, when accurate data are desired, it is advisable to assay a feed both before and after supplementation with DL-methionine. If an unsupplemented diet is not available to serve as a control, a correction factor is usually applied by assuming the presence of 0.005% methionine in the sample. This factor represents the average value found in over 20 different feeds assayed in this laboratory.

Microbiological Response of DL-Methionine *vs.* L-Methionine. When assayed with *S. faecalis*, DL-methionine shows an activity about 10% greater than calculated on the basis of its L-methionine content. Since L-methionine is used as the standard against which the activity of the DL-methionine is measured, the 10% increased response is due to the D-isomer. Although Stokes and coworkers (*3*) did not observe this activity of D-methionine, Steele and coworkers (*2*), using *Leuconostoc mesenteroides*, also found a small response from D-methionine when testing DL-methionine.

Calculation of Added DL-Methionine. The amount of DL-methionine present in a commercial feed is calculated as follows:

The amount of L-methionine determined by the microbiological assay procedure is adjusted for the amount of methionine or methionine-like material which occurs in the feed prior to supplementation with DL-methionine, and for the 10% of the D-methionine which was determined in the microbiological assay as L-methionine. For example, analysis of the first diet shown in Table II gave a value of 0.037% of L-methionine. The next column indicates that the control diet contained 0.010% of free methionine, thus reducing the amount of L-methionine response due to the added DL-methionine to 0.027%. This constitutes 110% of the L-methionine added, corresponding to 0.049% DL-methionine added to the diet. Since 0.05% DL-methionine of commercial grade (98% purity) was added originally, it is evident that this assay represents an excellent recovery of the added DL-methionine.

Applications. Many commercial feeds are prepared with certain ingredients which could possibly interfere with a microbiological determination of methionine. The assay procedure has been tested with diets containing a number of antibiotics, a coccidiostat, arsanilic acid, diphenyl-*p*-phenylenediamine (DPPD), and animal fat. These supplements were present in the amounts usually added to poultry diets but caused no interference with the accuracy of the assay, as is evident from the data shown in Table I.

Discussion. The method described has been used as a routine procedure for several years. The results are reproducible and the accuracy is considered satisfactory. The method is sensitive enough to detect as little as 0.0125% (0.25 pound of DL-methionine per ton of feed). Smaller amounts are not generally used for supplementation of animal feeds.

The principle of this method, water extraction of the supplemented feed, should be applicable to any added amino acid. The determination of L-lysine hydrochloride in animal feeds has been successfully carried out in this laboratory using a similar procedure.

The assays shown in Tables I and II were performed simultaneously with assays for total methionine content and therefore the use of L-methionine as the standard was indicated. However, if diets were tested solely for the presence of added DL-methionine, the response of a DL-methionine standard would

simplify interpretation. A microorganism responding equally well to DL- and L-methionine might be helpful. This problem merits further investigation.

Stability of DL-Methionine Added to Feeds

The stability of DL-methionine in poultry diets has been investigated under several conditions, as shown in Table II. Broiler rations supplemented with 0.05 and 0.0625% DL-methionine were kept at room temperature for as long as 13 months without deterioration. In another experiment a commercial broiler mash containing 0.05% DL-methionine was stored for 14 months under the roof of a warehouse in different types of containers. During the summer months the heat in this warehouse occasionally reached 110° F. (43.3° C.), but no loss of added DL-methionine was noted.

The effect of higher temperatures was also studied. For this purpose a commercial diet was supplemented with 0.05% DL-methionine and samples were subjected to temperatures up to 100° C. for varying periods of time. The results of this experiment, shown in Table II, indicate that when the diet was heated to 50° C. for 5 days, no loss of DL-methionine occurred. But when the temperature was raised to 100° C., considerable loss took place after 3 days. As the diet itself became brown and decomposed, the loss of methionine is not surprising.

Literature Cited

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CEREAL COMPONENTS

Free Amino Acids of Fresh and Aged Parboiled Rice

IRVING R. HUNTER, ROBERT E. FERREL, and DAVID F. HOUSTON

Western Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Albany 10, Calif.

Eighteen free amino acids were identified by filter-paper chromatography of adsorption-dialysis extracts of both fresh and oven-aged parboiled rice. Those in greatest initial concentration were alanine, aspartic acid, and glutamic acid; those in intermediate concentration were arginine, asparagine, glycine, leucine(s), lysine, proline, serine, valine, and one unidentified ninhydrin-reacting compound; those in lowest concentration were cystine, histidine, methionine, phenylalanine, threonine, tryptophan, and tyrosine. Decreases in size and intensity of amino acid spots from the aged rice indicated significant losses of amino acids during accelerated storage.

THE MARKED DARKENING OF PARBOILED RICE during accelerated storage (*5*) is believed to be the result of nonenzymatic browning reactions (*4*) in which amino groups and reducing sugars are known generally to take part. The parboiling process consists in steeping the rice in warm water, steaming under pressure, drying while still in the hull, and milling to remove hulls and bran. This treatment is known to increase the content of vitamins, minerals, phosphorus compounds, and reducing sugars in the milled product (*8*).

The present investigation of the content of—and changes in—free amino acids is part of a more comprehensive program to determine changes in parboiled rice during storage, and to develop means for accelerated testing of storage life. It has been established in prior work (*15*) that during storage both reducing sugars and total amino nitrogen decrease. The latter includes amino nitrogen from amino acids, the free amino groups of proteins, and possibly other compounds.

This report establishes the individual free amino acids present in the rice and shows that some of them decrease during storage. The results are preliminary to measurement of quantitative changes. No comparable determination appears to have been made on domestic varieties of rice. A report on amino acids of Indian parboiled rice appeared (*11*) when this work was nearly finished. A somewhat comparable investigation (*9*) was made in Japan on rice enriched with vitamin B_1 through a process which was essentially parboiling of white or brown rice instead of rough rice. The relation of these findings to the present results will be discussed following the experimental details.

Materials and Methods

The rice used was commercially parboiled Caloro, a short-grain variety, from the 1952 California crop. One portion was used as received, and a second was stored 28 days at 82° C. for accelerated aging.

To obtain the free amino acids, a 500-gram lot of each rice (ground to pass 20-mesh) was extracted by the adsorption dialysis technique of Hunter and coworkers (*6*). Each solution obtained was concentrated first under vacuum and then by storage over phosphorus pentoxide in an evacuated desiccator, to a volume of less than 5 ml. The concentrated solution was filtered, adjusted to 5 ml., and used for the chromatographic studies.

Amino acids were detected by two-dimensional ascending paper chromatography with the apparatus and techniques described by Hunter, Houston, and Owens (*7*). Chromatograms were run 16 hours at 27° C. on 9 × 9 inch sheets of either S&S 507 or S&S 589 (blue ribbon) papers.

Three solvent systems were utilized in these studies: (A) methanol-water-pyridine (80/20/4) (*12*), (B) phenol-water-ammonia (35/10, plus 0.3% ammonia in a beaker), and (C) *tert*-butyl alcohol–methyl ethyl ketone–water-diethylamine (40/40/20/4) (*12*). Proportions are by volume for solvent systems A and C, by weight for solvent system B. The solvent pairs employed were A-